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MRID No. 444577-33

DATA EVALUATION RECORD
§ 72-3 - ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK SHELL DEPOSITION STUDY

1. **CHEMICAL:** Prohexadione Calcium — PC Code No.: 112600

2. **TEST MATERIAL:** BAS 125 W — Purity: 90.6%

3. **CITATION:**

Author:	W.C. Graves, J.P. Swigert, and C.M. Holmes
Title:	BAS 125 W: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)
Study Completion Date:	April 14, 1997
Laboratory:	Wildlife International Ltd., Easton, MD
Sponsor:	BASF Corporation, Agricultural Products, Research Triangle Park, NC
Laboratory Report ID:	147A-148
MRID No.:	444577-33
DP Barcode:	D245631

4. **REVIEWED BY:** Karl Bullock, M.S., Environmental Scientist, Golder Associates Inc.

Signature: Karl Bullock **Date:** 7/7/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

Signature: P. Kosalwat **Date:** 7/7/98

5. **APPROVED BY:**

Signature: R. Chasow **Date:** 11/17/98

6. **STUDY PARAMETERS:**

Age or Size of Test Organism:	Mean valve height 40 mm
Definitive Test Duration:	96 hours
Study Method:	Static-renewal
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:** The study is scientifically sound and fulfills the guideline requirements for a mollusk shell deposition study. Based on mean measured concentrations, the EC_{50} was estimated to be >117 ppm ai, which classifies BAS 125 W as practically non-toxic to the Eastern oyster. The NOEC was determined to be 117 ppm ai.

Results Synopsis

EC_{50}: >117 ppm ai 95% C.I.: N/A

NOEC: 117 ppm ai Probit Slope: N/A

8. **ADEQUACY OF THE STUDY:**

A. Classification: Core

B. Rationale: Although a flow-through system was not used in this test, test solutions were aerated and renewed daily. Algal suspension was provided as a supplemental food and control growth was adequate (>2.0 mm).

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS:**

1. The amount of peripheral shell removed prior to testing was not reported.

2. The test was conducted using a static-renewal method; the guidelines require a flow-through test.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster (*Crassostrea gigas*) and the Eastern oyster (*Crassostrea virginica*)	*Crassostrea virginica*
Mean valve height 25 - 50 mm along the long axis	40 mm (Range: 27 - 47 mm)
Supplier	World's End Aquaculture, Queenstown, MD
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Oysters were of similar age

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days after collection	10 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Amount of peripheral shell growth removed prior to testing	Not reported
Feeding during the acclimation Must be fed to avoid stress.	Algae (*Isochrysis* sp., *Thalassiosira* sp., *Skeletonema* sp., and *Chaetoceros* sp.)
Pretest Mortality <3% mortality 48 hours prior to testing	Not reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural unfiltered seawater from Indian River Inlet, Delaware, diluted to a salinity of approximately 20‰ with well water.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ salinity, weekly range < 6%	20‰

Guideline Criteria	Reported Information
Water Temperature 15°-30° C, consistent in all test vessels	21.0 - 22.5°C
pH	7.9 - 8.2
Dissolved Oxygen ≥ 60% throughout	≥83% of saturation throughout the test
Total Organic Carbon	1.4 mg/L
Test Aquaria Should be constructed of glass or stainless steel.	57-L glass aquaria with 40 L of test solution.
Type of Dilution System Must provide reproducible supply of toxicant	N/A
Flow rate Consistent flow rate	Test solutions were gently aerated and renewed daily. Oysters were fed an algal suspension.
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Not reported
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L	Solvent: None Maximum conc.: N/A

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 oysters, then no definitive test is required.	Yes; 0.97, 3.2, 11, 36, and 120 mg ai/L resulting in shell growth reductions of 37, 11, 3, 24, and 18%, respectively.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	Negative control, 16, 26, 43, 72, and 120 mg ai/L.
Number of Test Organisms Minimum 20 individual per test level and in each control	20 oysters per treatment and control
Test organisms randomly or impartially assigned to test vessels?	Not reported
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	Temperature was measured in each test chamber at test initiation and termination and continuously in the dilution water control chamber. DO and pH were measured at test initiation, prior to and after each renewal (old and new solutions), and at test termination.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes; mean recoveries ranged from 96 to 100% of nominal.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes

Guideline Criteria	Reported Information
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0% in the controls and test concentrations
Control Shell Deposition Must be at least 2 mm.	Negative control: 2.10 mm
Recovery of Chemical	96-100%
Raw data included?	Yes
Signs of toxicity (if any) were described?	No sublethal signs of toxicity were observed.

Shell Growth

Concentration (mg ai/L)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Decrease[a]
Nominal	Mean Measured				
Control	>0.05	20	0	2.10 ± 0.83	-
16	16	20	0	2.15 ± 0.93	-2.4
26	25	20	0	2.25 ± 0.69	-7.1
43	42	20	0	1.92 ± 1.00	8.6
72	71	20	0	2.28 ± 0.90	-8.6
120	117	20	0	2.02 ± 0.85	3.8

[a]Compared to the solvent control. A negative sign indicates stimulation.

Other Significant Results: No sublethal signs of test material toxicity were observed. All test solutions appeared clear and colorless with the exception of the 120 mg ai/L test solution, which appeared slightly cloudy.

B. Statistical Results

Method: Non-parametric analysis of variance (Kruskal-Wallis ANOVA by ranks).

96-hr EC_{50}: >117 mg ai/L 95% C.I.: Not determined
Probit Slope: N/A NOEC: 117 mg ai/L

13. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Statistical Method for EC_{50}	Non-linear regression
EC_{50}	>117 ppm ai
Probit Slope	N/A
Statistical Method for NOEC	Dunnett's Test
NOEC	117 ppm ai

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for a mollusk shell deposition study. Based on mean measured concentrations, the EC_{50} was determined to be >117 ppm ai, which classifies BAS 125 W as practically non-toxic to Eastern oysters. The NOEC was determined to be 117 ppm ai. This study is classified as **Core.**

BAS 125 W SHELL DEPOSITION WITH EASTERN OYSTER

11:06 Wednesday, June 17, 1998

OBS	CONC	LOG_CONC	REP	Y
1	0	.	1	2.55
2	0	.	2	0.75
3	0	.	3	2.60
4	0	.	4	1.80
5	0	.	5	1.95
6	0	.	6	2.95
7	0	.	7	1.45
8	0	.	8	0.00
9	0	.	9	2.90
10	0	.	10	1.70
11	0	.	11	3.15
12	0	.	12	0.95
13	0	.	13	2.45
14	0	.	14	2.05
15	0	.	15	2.00
16	0	.	16	2.55
17	0	.	17	3.10
18	0	.	18	1.85
19	0	.	19	2.50
20	0	.	20	2.80
21	16	1.20412	1	3.40
22	16	1.20412	2	1.70
23	16	1.20412	3	1.40
24	16	1.20412	4	2.65
25	16	1.20412	5	2.25
26	16	1.20412	6	3.00
27	16	1.20412	7	0.00
28	16	1.20412	8	0.45
29	16	1.20412	9	1.90
30	16	1.20412	10	2.50
31	16	1.20412	11	2.35
32	16	1.20412	12	2.70
33	16	1.20412	13	0.70
34	16	1.20412	14	2.95
35	16	1.20412	15	3.10
36	16	1.20412	16	2.05
37	16	1.20412	17	1.85
38	16	1.20412	18	2.35
39	16	1.20412	19	2.40
40	16	1.20412	20	3.25
41	25	1.39794	1	2.75
42	25	1.39794	2	1.80
43	25	1.39794	3	0.55
44	25	1.39794	4	3.40
45	25	1.39794	5	2.40
46	25	1.39794	6	1.75
47	25	1.39794	7	1.45
48	25	1.39794	8	2.70
49	25	1.39794	9	3.20
50	25	1.39794	10	2.20
51	25	1.39794	11	2.45
52	25	1.39794	12	2.60
53	25	1.39794	13	2.55
54	25	1.39794	14	2.75
55	25	1.39794	15	1.80
56	25	1.39794	16	1.55
57	25	1.39794	17	2.65
58	25	1.39794	18	1.60
59	25	1.39794	19	1.80
60	25	1.39794	20	2.95
61	42	1.62325	1	0.80
62	42	1.62325	2	2.05
63	42	1.62325	3	1.20
64	42	1.62325	4	0.65

65	42	1.62325	5	3.05
66	42	1.62325	6	1.70
67	42	1.62325	7	2.55

BAS 125 W SHELL DEPOSITION WITH EASTERN OYSTER

11:06 Wednesday, June 17, 1998

OBS	CONC	LOG_CONC	REP	Y
68	42	1.62325	8	2.60
69	42	1.62325	9	1.80
70	42	1.62325	10	0.00
71	42	1.62325	11	2.70
72	42	1.62325	12	2.95
73	42	1.62325	13	0.00
74	42	1.62325	14	1.60
75	42	1.62325	15	2.55
76	42	1.62325	16	2.40
77	42	1.62325	17	1.15
78	42	1.62325	18	2.80
79	42	1.62325	19	2.65
80	42	1.62325	20	3.20
81	71	1.85126	1	1.65
82	71	1.85126	2	0.95
83	71	1.85126	3	2.50
84	71	1.85126	4	2.85
85	71	1.85126	5	3.45
86	71	1.85126	6	3.20
87	71	1.85126	7	3.05
88	71	1.85126	8	3.60
89	71	1.85126	9	0.90
90	71	1.85126	10	2.55
91	71	1.85126	11	1.50
92	71	1.85126	12	2.60
93	71	1.85126	13	1.85
94	71	1.85126	14	1.40
95	71	1.85126	15	0.45
96	71	1.85126	16	1.90
97	71	1.85126	17	2.50
98	71	1.85126	18	2.90
99	71	1.85126	19	3.00
100	71	1.85126	20	2.75
101	117	2.06819	1	0.65
102	117	2.06819	2	1.20
103	117	2.06819	3	1.80
104	117	2.06819	4	2.20
105	117	2.06819	5	2.95
106	117	2.06819	6	1.75
107	117	2.06819	7	0.00
108	117	2.06819	8	0.85
109	117	2.06819	9	2.60
110	117	2.06819	10	3.20
111	117	2.06819	11	2.45
112	117	2.06819	12	2.75
113	117	2.06819	13	1.50
114	117	2.06819	14	1.90
115	117	2.06819	15	2.00
116	117	2.06819	16	2.80
117	117	2.06819	17	2.45
118	117	2.06819	18	2.90
119	117	2.06819	19	1.75
120	117	2.06819	20	2.70

BAS 125 W SHELL DEPOSITION WITH EASTERN OYSTER

MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)

WEIGHTED REGRESSION

11:06 Wednesday, June 17, 1998

Non-Linear Least Squares Iterative Phase

Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC50	SIGMA	C0	Weighted SS

0	4.646000	1.522000	2.100000	43.482448
1	2.376200	7.9146759E-18	2.103189	42.223671
2	2.376200	7.9146759E-18	2.118750	41.899853
3	2.376200	7.9146759E-18	2.118750	41.899853

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	1	254.25000000	254.25000000
Residual	119	41.89985251	0.35209960
Uncorrected Total	120	296.14985251	
(Corrected Total)	119	41.89985251	

NOTE: The (approximate) Hessian is singular.

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC50	2.376200430	0.00000000000	2.3762004297	2.3762004297
SIGMA	0.000000000	0.00000000000	0.0000000000	0.0000000000
C0	2.118750000	0.07884642401	1.9626250075	2.2748749925

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	C0
LOG_EC50	.	.	.
SIGMA	.	.	.
C0	.	.	1

BAS 125 W SHELL DEPOSITION WITH EASTERN OYSTER
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
SUMMARY OF NONLINEAR REGRESSION
11:06 Wednesday, June 17, 1998

OBS	CONC	LOG_EC50	SIGMA	C0	RESID_SS	EC50
1	0	2.37620	7.9147E-18	2.11875	41.8999	237.794

BAS 125 W SHELL DEPOSITION WITH EASTERN OYSTER
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
WEIGHTED REGRESSION
11:06 Wednesday, June 17, 1998

Non-Linear Least Squares Iterative Phase
Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC25	SIGMA	C0	Weighted SS
0	3.626000	1.522000	2.100000	43.468697
1	2.738397	0.445974	2.102236	42.364424
2	2.362926	0.206680	2.102648	42.321921
3	2.103000	0.026673	2.102685	42.341046
4	2.103000	0.038335	2.138500	41.793258
5	2.103450	0.038335	2.138500	41.783527
6	2.103455	0.038335	2.138500	41.783437
7	2.103455	0.038335	2.138500	41.783437

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	2	254.25000000	127.12500000
Residual	118	41.78343697	0.35409692
Uncorrected Total	120	296.03343697	

(Corrected Total)	119	41.89817529		

NOTE: The (approximate) Hessian is singular.

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC25	2.103454690	0.03303372640	2.0380384216	2.1688709589
SIGMA	0.038335500	0.00000000000	0.0383355000	0.0383355000
C0	2.138500000	0.08701932376	1.9661767388	2.3108232612

Asymptotic Correlation Matrix

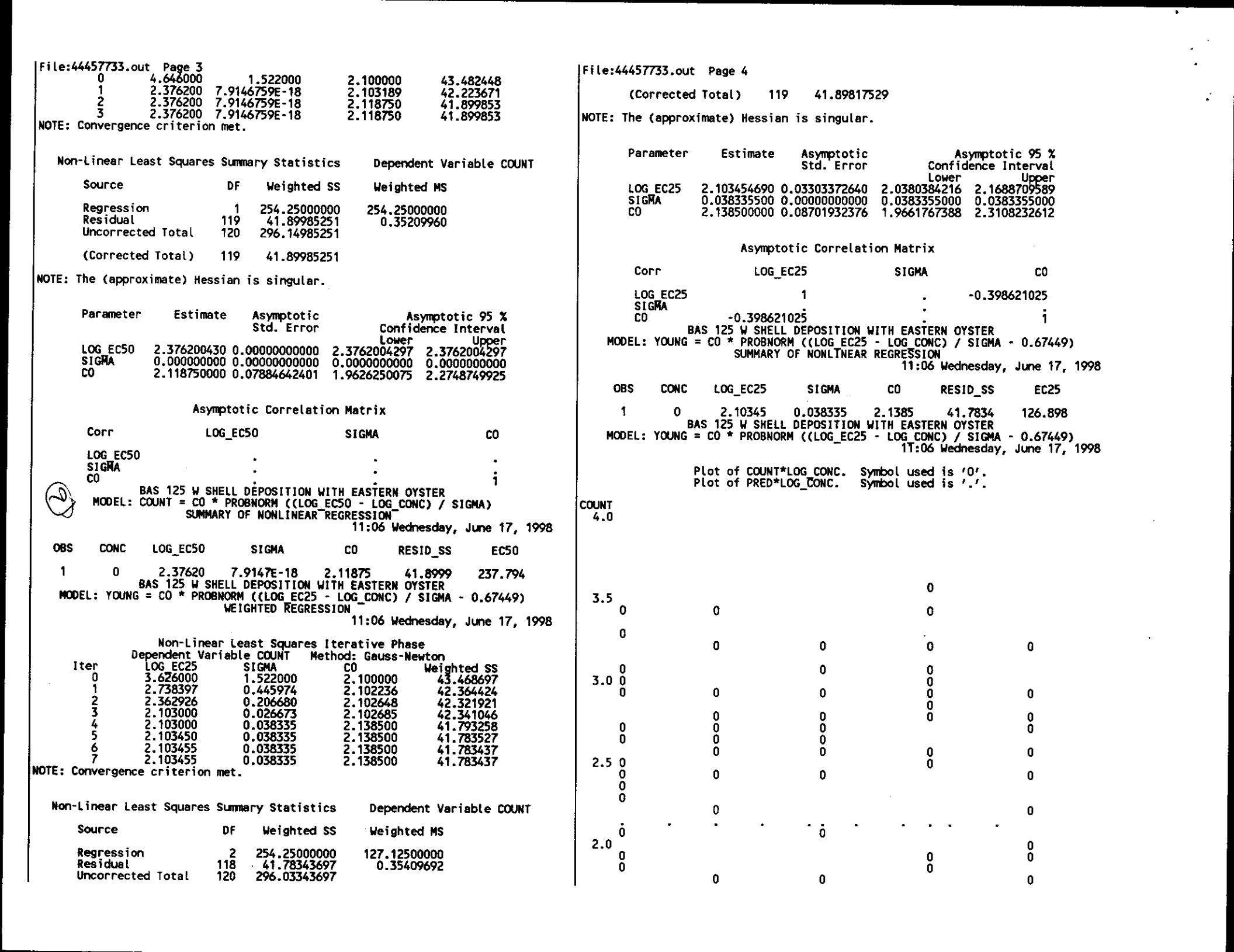
Corr	LOG_EC25	SIGMA	C0
LOG_EC25	1	.	-0.398621025
SIGMA	.	.	.
C0	-0.398621025	.	1

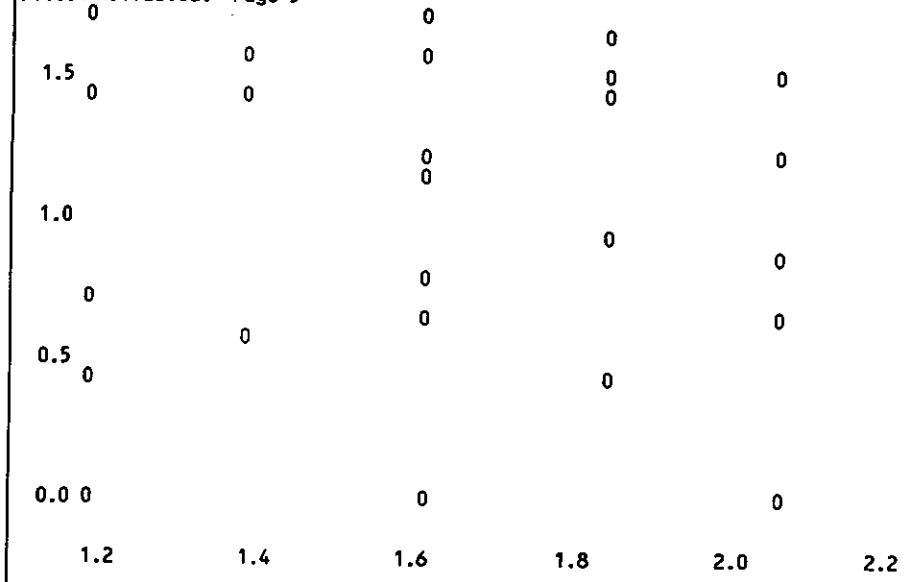
BAS 125 W SHELL DEPOSITION WITH EASTERN OYSTER
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
SUMMARY OF NONLINEAR REGRESSION
11:06 Wednesday, June 17, 1998

OBS	CONC	LOG_EC25	SIGMA	C0	RESID_SS	EC25
1	0	2.10345	0.038335	2.1385	41.7834	126.898

BAS 125 W SHELL DEPOSITION WITH EASTERN OYSTER
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
11:06 Wednesday, June 17, 1998

Plot of COUNT*LOG_CONC. Symbol used is '0'.
Plot of PRED*LOG_CONC. Symbol used is '.'.





NOTE: 49 obs had missing values. 117 obs hidden.

BAS 125 W SHELL DEPOSITION WITH EASTERN OYSTER
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
11:06 Wednesday, June 17, 1998

General Linear Models Procedure
Class Level Information

Class	Levels	Values
DOSE	6	0 16 25 42 71 117

Number of observations in data set = 120

BAS 125 W SHELL DEPOSITION WITH EASTERN OYSTER
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
11:06 Wednesday, June 17, 1998

General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1.82968750	0.36593750	0.48	0.7907
Error	114	86.94562500	0.76268092		
Corrected Total	119	88.77531250			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.020610	41.21846	0.873316	2.118750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	5	1.82968750	0.36593750	0.48	0.7907

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	5	1.82968750	0.36593750	0.48	0.7907

BAS 125 W SHELL DEPOSITION WITH EASTERN OYSTER
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
11:06 Wednesday, June 17, 1998

General Linear Models Procedure

Level of DOSE	N	RESPONSE Mean	RESPONSE SD
0	20	2.10250000	0.83405714
16	20	2.14750000	0.92927179
25	20	2.24500000	0.69412952
42	20	1.92000000	0.99768152
71	20	2.27750000	0.90036177
117	20	2.02000000	0.85384579

BAS 125 W SHELL DEPOSITION WITH EASTERN OYSTER
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
11:06 Wednesday, June 17, 1998

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESPONSE

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 114 MSE= 0.762681
Critical Value of Dunnett's T= 2.260
Minimum Significant Difference= 0.6241

Comparisons significant at the 0.05 level are indicated by '***'.

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
71 - 0	-0.4491	0.1750	0.7991
25 - 0	-0.4816	0.1425	0.7666
16 - 0	-0.5791	0.0450	0.6691
117 - 0	-0.7066	-0.0825	0.5416
42 - 0	-0.8066	-0.1825	0.4416

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING EC VALUES
Version 1.4

BAS 125 W Oyster Deposition

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
16.0000	100	0	0.0000	0.0000	0.0119
25.0000	100	0	0.0000	0.0000	0.0164
42.0000	100	9	0.0900	0.0900	0.0235
71.0000	100	0	0.0000	0.0000	0.0332
117.0000	100	4	0.0400	0.0400	0.0452

Chi - Square Heterogeneity = 25.612

****
* WARNING *
*
* Significant heterogeneity exists. The results reported *
* for this data set may not be valid. The results should *
* be interpreted with appropriate caution. *
****

****
* NOTE *
*
* Slope not significantly different from zero. *
* EC fiducial limits cannot be computed. *
****

Mu = 4.646003
Sigma = 1.522005

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	1.947446	2.059623	(-4.606275,	8.501166)
Slope	0.657028	1.173424	(-3.076806,	4.390862)

Theoretical Spontaneous Response Rate = 0.0000

BAS 125 W Oyster Deposition

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	12.7457		
EC 5.00	138.8216		
EC10.00	495.9086		
EC15.00	1171.1090		
EC50.00	44259.1680		
EC85.00	1672665.8800		
EC90.00	3950074.5000		
EC95.00	14110730.0000		
EC99.00	%153689616.0000		

EC_{25} = 4229.212

BAS 125 W Oyster Deposition

PLOT OF ADJUSTED PROBITS AND PREDICTED REGRESSION LINE